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Associations of Perfluoroalkyl Substances (PFASs) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK)

Marc-André Verner[1,2], Anne E. Loccisano[3], Nils-Halvdan Morken[4,5], Miyoung Yoon[3], Huali Wu[3], Robin McDougall[6], Mildred Maisonet[7], Michele Marcus[8], Reiko Kishi[9], Chihiro Miyashita[9], Mei-Huei Chen[10], Wu-Shiun Hsieh[10], Melvin E. Andersen[3], Harvey J. Clewell III[3], and Matthew P. Longnecker[11]

[1]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [3]The Hamner Institutes for Health Sciences, Center for Human Health Assessment, Research Triangle Park, North Carolina, USA; [4]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway; [5]Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway; [6]Aegis Technologies, Huntsville, Alabama, USA; [7]Biostatistics and Epidemiology Department, College of Public Health, East Tennessee State University, Johnson City, Tennessee, USA; [8]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA; [9]Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan; [10]Department of Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; [11]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, DHHS, Research Triangle Park, North Carolina, USA

Address correspondence to Marc-André Verner, Department of Occupational and Environmental Health, School of Public Health, Université de Montréal, 2375 chemin de la Cote-Sainte-Catherine, Suite 4105, Montréal, Quebec, Canada H3T 1A8. Telephone: 1-514-343-6465. E-mail: verner.marcandre@gmail.com

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Abstract

Background: Prenatal exposure to perfluoroalkyl substances (PFAS) has been associated with lower birth weight in epidemiologic studies. This association could be attributable to glomerular filtration rate (GFR) which is related to PFAS concentration and birth weight.

Objectives: To use a physiologically based pharmacokinetic (PBPK) model of pregnancy to assess how much of the PFAS-birth weight association observed in epidemiologic studies might be attributable to GFR.

Methods: We modified a PBPK model to reflect the association of GFR with birth weight (estimated from three studies of GFR and birth weight) and used it to simulate PFAS concentrations in maternal and cord plasma. The model was run 250,000 times, with variation in parameters, to simulate a population. Simulated data were analyzed to evaluate the association between PFAS levels and birth weight due to GFR. We compared simulated estimates to those from a meta-analysis of epidemiologic data.

Results: The reduction in birth weight for each 1 ng/ml increase in simulated cord plasma for perfluorooctane sulfonate (PFOS) was 2.72 g (95% CI: -3.40, -2.04), and for perfluorooctanoic acid (PFOA) was 7.13 g (95% CI: -8.46, -5.80); results based on maternal plasma at term were similar. Results were sensitive to variations in PFAS level distributions and the strength of the GFR-birth weight association. In comparison, our meta-analysis of epidemiologic studies suggested that each 1 ng/ml increase in prenatal PFOS and PFOA levels was associated with 5.00 g (95% CI: -21.66, -7.78) and 14.72 g (95% CI: -8.92, -1.09) reductions in birth weight.

Conclusion: Results of our simulations suggest that a substantial proportion of the association between prenatal PFAS and birth weight may be attributable to confounding by GFR and that confounding by GFR may be more important in studies with sample collection later in pregnancy.

Introduction

Perfluoroalkyl substances (PFAS) are synthetic compounds that are resistant to degradation and have been found worldwide in environmental media and biota, including humans. The most widely studied PFAS are perfluorooctane sulfonate (PFOS) and perfluorooctanoaic acid (PFOA). PFOS was an ingredient in the Scotchgard stain repellent manufactured by 3M, but the company decided to stop producing PFOS and related compounds in 2002 after it had been found in wildlife and humans (3M 2000). PFOA is a surfactant that is used in the production of many consumer goods, including nonstick coating in cookware. The eight major companies producing or using PFOA have agreed to work toward eliminating emissions and product content of PFOA by 2015 (PFOA Stewardship Program 2006). Despite the reductions in the production and emission of PFOS and PFOA, these persistent compounds can still be detected in biological samples from the general population. For example, PFOS and PFOA have been detected in the blood of more than 98% of participants in the 2009-2010 National Health and Nutrition Examination Survey (NHANES) (CDC 2013) and 2009-2011 Canadian Health Measure Survey (CHMS) (Health Canada 2013). PFOS and PFOA have also been detected in maternal blood during pregnancy, cord blood at delivery and breast milk (SK Kim et al. 2011; Olsen et al. 2009), indicating that humans are exposed during critical prenatal and early postnatal windows of development.

Many epidemiologic studies have reported an association between maternal and cord blood PFAS levels and reductions in birth weight (Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2007; Maisonet et al. 2012; Washino et al. 2009; Whitworth et al. 2012). Although these studies accounted for potential confounding by many variables, none adjusted for glomerular filtration rate (GFR). GFR, the flow rate of fluid being filtrated by the kidneys, increases by about 50%

during the first half of pregnancy and declines slightly during the second half of pregnancy (Gibson 1973). Two studies of GFR during pregnancy have shown that women whose GFR fails to rise sufficiently during pregnancy tend to have smaller babies (Gibson 1973; Morken et al. 2014). On the other hand, GFR is likely to influence the urinary excretion of xenobiotics like PFAS. Indeed, higher blood PFAS levels have been observed in people with lower GFR (Shankar et al. 2011; Watkins et al. 2013). Watkins et al. (2013) evaluated the direction of the association between PFOA and reduced kidney function (indicated by GFR) by comparing results obtained with measured serum PFOA levels (which could be influenced by GFR) and estimated serum PFOA levels (which were independent of GFR): an association was only observed with measured PFOA, suggesting the association may be a consequence of, rather than a cause of, decreased kidney function. If so, women with lower GFR during pregnancy would tend to have smaller babies and higher blood PFAS levels. This raises the possibility that GFR confounds the association between prenatal PFAS exposure and birth weight. To what extent GFR influences this association has yet to be evaluated.

In this study, we assessed how much of the epidemiologic association between prenatal PFOS and PFOA (PFAS thereafter) exposure and birth weight could be attributable to confounding by GFR. We modified a recently developed physiologically based pharmacokinetic (PBPK) model of PFAS during pregnancy (Loccisano et al. 2013) to reflect the association between GFR and PFAS levels and birth weight. The model was run repeatedly, using Monte Carlo simulation techniques, with variation in parameters, to simulate a population. Estimates of the birth weight-PFAS association obtained from simulated PFAS levels and birth weight were subsequently compared to estimates from a meta-analysis of existing epidemiologic studies.

Methods

Overview

We used a PBPK model to run Monte Carlo simulations of a study population, and generate pairs of predictions for PFAS level and birth weight. PBPK-derived estimates were subsequently analyzed by linear regression. We also performed a meta-analysis of published epidemiologic studies of prenatal PFAS exposure and birth weight to obtain summary effect estimates. Results obtained from simulated PFAS levels and birth weights were compared to results from our meta-analysis to evaluate how much of this association might be attributable to the influence of GFR.

The PBPK model

We modified a published PBPK model of PFOA and PFOS during pregnancy (Loccisano et al. 2013). This multi-compartment model included maternal compartments (plasma, liver, fat, gut, skin, mammary, rest of body, kidney, filtrate and storage) and the placenta, fetal plasma, rest of fetal body, and amniotic fluid (Figure 1). Exposure to PFAS was modeled as an input into the maternal plasma compartment to encompass absorbed doses through different routes. Distribution in the different compartments was driven by blood flow rates in and out of compartments, tissue volume, and tissue:blood partition coefficients. PFAS excretion in urine was modeled as a multi-step process: the free (unbound) PFAS in plasma was first filtered through the kidneys followed by extensive active reabsorption, with the unreabsorbed fraction continuing its way to a storage compartment prior to excretion. We updated the description of placental blood flow and fetal cardiac output according to equations presented in Yoon et al. (2011). The modified version of the PBPK model code is provided in Supplemental Material. A

conceptual representation with basic mass-balance differential equations is also provided in Supplemental Material (see Figure S1).

We also modified the model so that the initial body burden (at the beginning of pregnancy) and intake rate during pregnancy are calculated based on an initial plasma PFAS level ($C_{initial}$ [ng/ml]). The initial amount of PFAS in the different maternal tissues ($Amount_t$) at each Monte Carlo simulation (i) was computed as the product of the initial plasma PFAS level ($C_{initial}$), the tissue:plasma partition coefficient ($Partition_t$) and the tissue volume ($Volume_t$):

$$Amount_{t(i)} = C_{initial(i)} \times Partition_{t(i)} \times Volume_{t(i)} \quad [1]$$

Maternal PFAS intake rate during pregnancy was estimated from initial plasma PFAS level. To estimate maternal PFAS intake rate during pregnancy, we assumed the initial plasma PFAS level to be at steady state. The hourly intake rate was calculated accordingly using a rearrangement of a classic steady state equation that accounts for compound-specific half-life (h), volume of distribution (l) and dosing interval (h) (Dhillon and Kostrzewski 2006):

$$Intake\ (ng/h)_{(i)} = C_{initial(i)} \times Volume\ of\ distribution_{(i)} \times Dosing\ interval \times \ln(2)/Half\text{-}life \quad [2]$$

where the Volume of distribution was calculated based on partition coefficients and organ volumes, the dosing interval was 1 h (simulation time increment), and the half-lives of PFOS and PFOA were 47,304 h (5.4 years) and 33,288 h (3.8 years) (Olsen et al. 2007).

To parameterize the relationship between GFR and birth weight, we performed a meta-analysis of three studies where individual-specific paired GFR and birth weight measurements were available in the publication or made available to us (Dunlop 1981; Gibson 1973; Morken et al. 2014). Other studies of GFR or indicators of GFR (e.g., serum creatinine, serum uric acid) and

birth weight were identified but did not report individual-specific data or regression coefficients and, consequently, could not be used in our meta-analysis (Akahori et al. 2012; Davison and Hytten 1974; Dunlop et al. 1978; Duvekot et al. 1995; Knopp et al. 1985; Laughon et al. 2009). Because GFR changes during pregnancy and the measurements were taken at different times during pregnancy, we calculated standardized GFR values (GFR_{ratio}) as the ratio of the observed GFR for each subject to the mean GFR at that gestational age (Gibson 1973 [28 gestational weeks]; Dunlop 1981 [26 gestational weeks]; Morken et al. 2014 [mean = 18 gestational weeks]). We computed the coefficient relating birth weight to GFR_{ratio} as the inverse-variance weighted average of the coefficient based on regression models of data from Gibson (1973) (n=20), Dunlop (1981) (n=25) and Morken et al. (2014) (n=953). The raw data from these studies was either presented in the original publication (Gibson 1973; Dunlop 1981) or was available to us (Morken et al. 2014). In the first two studies, GFR was measured using inulin clearance. In the third study, GFR was estimated based on plasma creatinine and the Cockroft-Gault formula (Koetje et al. 2011). A separate multiple regression model of birth weight was fitted for each study; all models were adjusted for gestational age at birth. The Morken et al. (2014) data were additionally adjusted for pre-pregnancy body weight and sampling strata. Because estimation of GFR on the basis of a single measure of plasma creatinine is known to be imprecise (Aras et al. 2012), the coefficient for GFR_{ratio} from the Morken et al. (2014) study was deattenuated to account for the effect of measurement error (Willett 1990), by dividing by an intraclass correlation coefficient of 0.76 for serum creatinine (Al-Delaimy et al. 2006) prior to calculating the overall inverse-variance weighted average. Each unit increase in GFR_{ratio} was associated with an increase in birth weight of 67 g (SE = 535) in the Dunlop (1981) study, 1603 g (SE = 784) in the Gibson (1973) study and 164 g (SE = 77) in the Morken et al. (2014) study.

The meta-analytic coefficient was 175.5 g (SE = 75.9) increase in birth weight per unit increase in GFR_{ratio}.

We used a 2-tier approach to generate variability in GFR_{ratio} and induce an association between GFR_{ratio} and birth weight in Monte Carlo simulations. For each Monte Carlo simulation (i), we first sampled a GFR_{ratio} value from the distribution of GFR_{ratio} in the data of Morken et al. (2014) (mean: 1.0; SD: 0.246; range: 0.508-1.492 [± 2 SDs]). The SD from Morken et al. (2014) was selected because in this more recent study, the distribution of GFR_{ratio} was considered to be more relevant, because of the increase in prevalence of overweight and obesity, and the correlation of GFR with body mass index (Bosma et al. 2004). During each simulation, the time-course of GFR (GFR_t) during pregnancy was obtained by multiplying the Reference gestational GFR_t profile (GFR as a function of time elapsed since conception, as described in the original PBPK model) by the sampled GFR_{ratio}:

$$GFR_{t(i)} = GFR_{ratio(i)} \times \text{Reference gestational } GFR_t \quad [3]$$

Then, we calculated a birth weight according to the meta-analytic regression between GFR_{ratio} and birth weight derived from three studies as described above. This was accomplished by using the equation derived from the aforementioned regression and randomly sampling an error term based on the distribution of residuals:

$$\text{Calculated birth weight } (g)_{(i)} = \text{Intercept} + \beta \times GFR_{ratio(i)} + \text{Residual}_{(i)} \quad [4]$$

where the intercept was 3,376 g, the β was 175.5 g per 1 unit increase in GFR_{ratio} and the residual was sampled from a distribution with a mean of 0 g, a SD of 441 g and ranging from -882 g to 882 g (±2 SDs). Fetal growth in the original PBPK model was described using a time-dependent

fetal growth curve (Loccisano et al. 2013). We adjusted this standard fetal growth curve to match the Calculated birth weight from Equation 4. To do so, we multiplied the standard fetal growth curve (Reference fetal $weight_t$) by the ratio of Calculated birth weight on the Reference fetal $weight_t$ at delivery (3,509 g). For each simulation (i), the time-course of fetal weight (Fetal $weight_t$) was described using the following equation:

$$\text{Fetal weight}_{t(i)} = (\text{Calculated birth weight}_{(i)}/3{,}509\text{ g}) \times \text{Reference fetal weight}_t \quad [5]$$

PBPK model global sensitivity analysis

Because the PBPK model used herein incorporates over 40 parameters that can vary within a population (e.g., volume of organs, perfusion rates, tissue:plasma partition coefficients), we first ran a sensitivity analysis to identify parameters with the highest relative influence on maternal plasma PFAS levels across pregnancy and cord plasma PFAS levels at delivery. We opted for the Morris global method which evaluates parameter sensitivity over a range of physiological scenarios by taking the mean of many local sensitivity analysis calculated over the entire parameter space, thus accounting for interactions (McNally et al. 2011). We allowed parameters to vary between 70% and 130% of their mean value, i.e., a 15% coefficient of variation with bounds at ± 2 SDs. For this exercise, we used initial maternal plasma levels of 13.02 ng/ml for PFOS and 2.53 ng/ml for PFOA to reflect levels in published epidemiologic studies as noted below in the *Monte Carlo simulations* section. Sensitivity coefficients were calculated by adapting the M code of the Morris Test included in the acslX Optimum suite of tools (Aegis Technologies Inc., Huntsville, AL, USA) to our study. The set of most influential parameters, those for which small perturbations have the most significant effect on PFOS and PFOA levels

(coefficient within a factor of 10 of the most sensitive model parameter at any month of pregnancy or at delivery) were allowed to vary in the Monte Carlo analyses.

Assessment of PBPK model accuracy

To assess how well the model describes the pharmacokinetics of PFAS during pregnancy, we compared simulated plasma PFAS profiles to observed serial levels. We identified two reports with data that were not used by Loccisano et al. (2013) for model development and met the following criteria: presented two serial maternal blood PFAS levels and presented sufficient information on sample collection times (Glynn et al. 2012; Monroy et al. 2008). For each of the two reports and each PFAS (PFOS and PFOA), we performed 10,000 Monte Carlo iterations. At each Monte Carlo iteration, the model i) sampled values for sensitive parameters identified in the global sensitivity (see Table 1), ii) sampled a plasma PFAS level from the published distributions at the first blood sample collection time point, iii) adjusted the initial plasma level (at the time of conception), by iterative model simulations, to obtain matching simulated and sampled PFAS level at the time of the first blood sample collection (tolerance: 0.1%) and iv) simulated a complete pharmacokinetic profile based on the initial plasma level. We visually compared the distribution of simulated plasma PFAS profiles from the Monte Carlo iterations to the distribution of observed PFAS levels in the second blood samples from the two reports mentioned above.

Monte Carlo simulation

We used a Monte Carlo procedure to simulate population PFOA and PFOS levels across pregnancy. At each Monte Carlo iteration, the PBPK model sampled values for sensitive parameters identified in the global sensitivity analyses and initial blood PFAS levels from

probabilistic distributions (see Table 1) prior to simulation of PFAS levels during the 9 months of pregnancy. To be able to compare results from simulations to those from epidemiologic studies on PFAS and birth weight included in our meta-analysis (described below in the *Meta-analysis of PFAS-birth weight epidemiologic studies* section, Apelberg et al. 2007; Chen et al. 2012; Fei et al. 2007; Hamm et al. 2010; Maisonet et al. 2012; Washino et al. 2009; Whitworth et al. 2012), we used initial plasma PFAS distributions based on levels reported in these studies. We calculated the mean PFOS (13.02 ng/ml) and PFOA (2.53 ng/ml) levels by averaging the reported mean or median maternal blood or cord blood levels (studies were weighted equally). These epidemiologic studies reported different measures of spread for blood PFAS levels (i.e., range, standard deviation, geometric standard deviation, interquartile range). Because these measures of spread cannot be directly combined, we derived a standard deviation based on coefficients of variations of 0.37 for PFOS and 0.45 for PFOA calculated using data from Fei et al. (2007), the largest study (n=1,399) included in our meta-analysis (described below). Monthly simulated maternal plasma PFAS levels, simulated cord plasma levels at delivery, and calculated birth weight were collected from simulations to be used in regression models of PFAS and birth weight. We ran 250,000 Monte Carlo iterations to achieve convergence in the PFAS-birth weight linear regression coefficient (β).

Sensitivity analyses

We evaluated the influence of different assumptions on the association between PBPK-derived PFAS levels and birth weight. In addition to analyses noted above, we ran multiple Monte Carlo simulations with different parameters for PFAS distributions (higher and lower means and standard deviations), different coefficients for the GFR-birth weight association. Specifically, we halved or doubled these three parameters, one at a time. We also ran Monte Carlo simulations

with different sampling seeds to evaluate reproducibility. We identified two studies that evaluated PFOA half-life in populations exposed through drinking water; the Brede et al. (2010) estimated a half-life of 3.26 years, which is similar to the 3.8-year half-life used in our study (Olsen et al. 2007), whereas Bartell et al. (2010) estimated a shorter half-life of 2.3 years. To evaluate the impact of a shorter half-life on our results, additional Monte Carlo simulations were carried out using the half-life reported by Bartell et al. (2010).

Meta-analysis of PFAS-birth weight epidemiologic studies

We identified human studies published in English in 2012 or earlier using the PubMed search terms birth weight and perfluorooctane sulfonate or perfluorooctanoic acid. This identified articles with the search terms in the title, abstract, or key words. To be eligible for inclusion in the analysis, the study had to have results available from a multiple regression model of birth weight (g) as a function of PFOS or PFOA in ng/ml concentration in maternal blood from pregnancy or cord blood. In one case (Apelberg et al. 2007), the β coefficient originally published (g birth weight per interquartile increase in PFAS) was reexpressed as per ng/ml by using the interquartile distance. In three instances we found studies that had fit models similar to what we sought, but the published results could not be reexpressed to obtain a reasonable approximation of what we needed. In these cases we contacted the original authors to obtain the coefficients of interest. Specifically, Washino et al. (2009) and Chen et al. (2012) had fit models with log of PFAS as the independent exposure variable, and Maisonet et al. (2012) had fit the desired model but had not put the β coefficients in the publication. We used these regression coefficients to calculate inverse-variance weighted summary β coefficients for PFOS and PFOA. A list of included and excluded studies and a brief description of each is provided in Table S1 of the Supplemental Material.

Results

PBPK modeling of PFAS levels

We first performed a Morris global sensitivity analysis to identify sensitive model parameters, where a higher coefficient means greater sensitivity. The following parameters had a sensitivity coefficient within a factor of 10 of the most sensitive parameter at some point during pregnancy or at delivery: pre-pregnancy body weight, liver volume, liver:plasma partition coefficient, rest of body:plasma partition coefficient, free fraction in maternal and cord plasma, renal reabsorption constant and maximum reabsorption velocity (sensitivity coefficients are presented in Table S2 of the Supplemental Material). For example, the most sensitive parameter for PFOS levels in cord plasma was the free fraction in fetal plasma (global sensitivity coefficient = 0.0046). In a one-at-a-time sensitivity analysis, a 10 % change in this parameter was associated with a 8.9 % change in simulated cord plasma PFOS level. In comparison, a 10 % change in the liver volume (global sensitivity coefficient = 0.0003) was associated with a 0.9 % change in simulated cord plasma PFOS level.

To assess model accuracy, we simulated maternal plasma PFAS levels based on the first of the two serial measurements of PFAS from two published studies (Glynn et al. 2012; Monroy et al. 2008) and visually compared simulated profiles to observed levels (Figure 2). Simulated and observed PFOS and PFOA levels declined over the course of pregnancy in a similar fashion. However, the model slightly underestimated the decline in PFOA levels from the Glynn et al. (2012) study: Mean simulated PFOA level at the time of second blood draw was 4.3 ng/ml whereas mean reported level was 4.0 ng/ml.

In linear regression analyses, the association between simulated maternal and cord plasma PFAS levels and birth weight was dependent on the time elapsed after conception. For both PFOA (Figure 3A) and PFOS (Figure 3B), the association between simulated maternal plasma levels and birth weight only appeared after the 3rd month of pregnancy and was strongest at the time of delivery. The association between simulated PFOA levels and birth weight was similar for maternal plasma at term (β: -7.9 g; 95% CI: -9.4, -6.4) and cord plasma (β: -7.1 g; 95% CI: -8.5, -5.8). For PFOS, the association between simulated cord plasma levels and birth weight (β: -2.7 g; 95% CI: -3.4, -2.0) was slightly stronger than that estimated based on simulated maternal plasma levels (β: -1.5 g; 95% CI: -1.8, -1.1).

In sensitivity analyses, we evaluated whether the results were robust to changes in initial plasma PFAS level distributions (mean and SD), variations in coefficients for the GFR-birth weight association and different Monte Carlo sampling seeds (reproducibility). These analyses showed that the strength of the simulated PFAS-birth weight association (i.e., confounding by GFR) is influenced by initial plasma PFAS level distributions and the GFR-birth weight coefficient: stronger associations were obtained with lower mean initial plasma PFAS levels and lower SDs, and with higher GFR-birth weight coefficients (Table 2). When more than one parameter was changed at a time, their influence was additive. As an example, a lower PFOA mean (multiplier = 0.5) and a stronger for the GFR-birth weight association (multiplier = 2) resulted in a 23.3 g (95% CI: -26.0, -20.6) decrease in birth weight per ng/ml increase in simulated cord plasma levels; conversely, a higher PFOA mean (multiplier = 2) and a weaker β for the GFR-birth weight association (multiplier = 0.5) resulted in a 2.4 g (95% CI: -3.1, -1.8) decrease in birth weight per ng/ml increase in simulated cord plasma levels. Results from Monte Carlo simulations using different sampling seeds did not vary substantially, which supports the

reproducibility of results (Table 2). Using a shorter half-life of 2.3 years for PFOA (compared with 3.8 in main analyses) increased the strength of the association between simulated levels in maternal plasma at term and birth weight by 21 % (β: -9.6 g; 95% CI: -11.0, -8.2) and between simulated levels in cord plasma and birth weight by 14 % (β: -8.1 g; 95% CI: -9.4, -6.8).

Meta-analysis of epidemiologic studies

All studies of prenatal PFOA reported an association with reduced birth weight, with β coefficients ranging from -2.1 g to -64.4 g per ng/ml increase in PFOA levels (Figure 3A). An association between PFOS and reduced birth weight was observed in 6 out of 7 studies, with β coefficients ranging from -13.0 g to -1.5 g per ng/ml increase in PFOS levels (Figure 3B). The summary β coefficients for g birth weight per ng/ml increase in PFOA and PFOS levels were -14.7 g (95% CI: -21.7, -7.8) and -5.0 g (95% CI: -8.9, -1.1), respectively.

Discussion

In this study, we aimed to evaluate how much of the epidemiologic association between prenatal exposure to PFAS and reduced birth weight might be attributable to confounding by GFR. Results from Monte Carlo PBPK model simulations suggest that GFR drives a portion of this association, but not all of it, and that its influence becomes more important with increasing gestational weeks.

When our default assumptions were applied, the association between simulated maternal and cord plasma PFAS levels at the time of delivery and birth weight represented a substantial proportion of the association observed in our meta-analysis of epidemiologic studies. This suggests that epidemiologic studies presented herein, which have not controlled for GFR, might

have overestimated the influence of prenatal exposure to PFAS on fetal growth. Our results also suggested that GFR had less influence on PFAS levels in maternal plasma early in pregnancy. In a meta-regression analysis of the epidemiologic data in Figures 3A and 3B that we conducted (not shown), week of blood draw was associated with a larger negative coefficient for PFOS (-0.39 g birth weight per ng/ml increase in PFOS per gestational week, $p < 0.01$). For PFOA, the corresponding coefficient was -0.006, $p = 0.98$. While the meta-regression results support our hypothesis for PFOS, the lack of support for PFOA could be due to the small number of studies included, and other sources of heterogeneity.

In light of these results, epidemiologic studies investigating the effects of prenatal PFAS on fetal growth should account for the influence of GFR. Different approaches could be considered. An option would be to sample maternal plasma before pregnancy or during the first trimester, when changes in GFR have not yet influenced PFAS significantly according to simulated results. Statistically adjusting for GFR estimated from plasma creatinine levels or cystatin C levels (Tidman et al. 2008) could also help reduce confounding by GFR. Another approach would be to use a PBPK model to simulate results that are specific to their study sample collection time and PFAS distribution. Assuming the PBPK model and key assumptions are valid, the contribution of GFR to the observed association could be inferred from a comparison of simulated vs. observed results. Two studies of communities with high exposure to PFOA have used PFOA serum levels estimated using one-compartment pharmacokinetic model coupled with a model for individual exposure to evaluate the association between prenatal exposure and birth outcomes (Savitz et al. 2012a; Savitz et al. 2012b). Because the PFOA level estimates were not based on biological levels, the association between estimated levels and birth outcomes cannot be confounded by GFR. Of note, these studies were not suggestive of an association between

prenatal PFOA exposure and birth weight (Savitz et al. 2012b). For example, in Savitz et al. (2012b), based on data for 4,534 births, the adjusted change in birth weight per 100 ng/ml increase in estimated serum PFOA was -15 g (95% CI -43, 14).

Our results also have implications with regards to future meta-analyses of prenatal PFAS and birth weight. As noted by Egger et al. (1998), the real strength of meta-analyses is to identify factors responsible for heterogeneity across studies. According to our simulations, the contribution of GFR to the association between simulated PFAS levels and birth weight is influenced by the timing of sample collection and PFAS level distribution (mean and SD). A meta-analysis, including a meta-regression, based on more studies, and consideration of other sources of heterogeneity, would be of interest.

Certain assumptions might have introduced bias in our study. Because individual-specific data on GFR, PFAS, and birth weight were not available, we could only evaluate the PBPK model validity on a population level. Should extensive individual-specific measurements be available during pregnancy, the model could be further calibrated and evaluated. Nevertheless, when we simulated plasma PFAS levels across pregnancy in women from two studies who had their blood levels measured twice, simulated levels followed a decline in PFAS levels that closely matched reported levels. Because the simulated association between PFAS and birth weight was shown to be sensitive to the distribution of PFAS levels, the strength of the association between simulated PFAS levels and birth weight from this study cannot be compared to epidemiologic studies or meta-analyses with a different distribution of plasma PFAS levels. The coefficient of the GFR-birth weight association used in the Monte Carlo simulation was also shown to be a sensitive parameter. Should the true association between GFR and birth weight be stronger or weaker than

the meta-analytic relation used in this study, one would expect the simulated association between PFAS and birth weight to change accordingly (i.e., a stronger GFR-birth weight association would increase the strength of the simulated PFAS-birth weight association and vice versa). We also did not account for the potential association between GFR and initial PFAS concentration at conception. For example, pre-pregnancy GFR was correlated with GFR during pregnancy in the Gibson (1973) study (r=0.55-0.69) and in the Dunlop et al. (1981) study (r=0.27-0.30), although correlations were only statistically significant in the Gibson (1973) study. If pre-pregnancy GFR is associated with GFR during pregnancy, we could have underestimated the portion of the PFAS-birth weight association that is attributable to GFR by not accounting for the relationship between GFR and initial PFAS level. Also, we did not account for correlations across model parameters in Monte Carlo simulations, a factor that may have increased the spread of simulated blood PFAS levels (Burmaster and Anderson 1994). The assumptions that the initial plasma PFAS level is at steady state and that PFAS intake on a body weight basis is constant throughout pregnancy may oversimplify variations that are expected to occur in reality.

The meta-analysis for PFOA that we did was based on data for over 4,000 subjects. The more formal meta-analysis by Johnson et al. (2014) included two additional studies, each with fewer than 50 subjects (Fromme et al. 2010; S Kim et al. 2011). In addition, the value we used to represent the data from the Washino et al. (2009) study was adjusted for more factors than was the one used by Johnson et al. (2014), and the value we used was closer to the null. Thus, the slightly more negative summary in Johnson et al. (2014) (-18.9 g/ng/ml) than in our study (-14.7 g/ng/ml) was probably due to the inclusion of the two additional studies and the different coefficient for the Washino et al. (2009) result. We regard the two meta-analyses as showing close agreement.

In a recent systematic review of the literature, Lam et al. (2014) concluded that there was sufficient evidence of an association between prenatal PFOA and fetal growth. Authors evaluated the hypothesis that GFR influences the PFOA-fetal growth association by reviewing the literature on GFR and fetal growth. They suggested that there is insufficient evidence for an association between maternal GFR during pregnancy and fetal growth, and they consequently rejected the hypothesis that GFR underlies the relationship between PFOA and fetal growth. However, Lam et al. (2014) did not include the study by Morken et al. (2014) in their systematic review of GFR and fetal growth, most likely because the results had not been published at the time. This new study by Morken et al. (2014), by far the largest to date (n=953), revealed a significant association between estimated GFR and birth weight. When considering all available studies on the subject, we found that large studies consistently demonstrated an association between estimated GFR or indicators of GFR (e.g., serum creatinine, serum uric acid) and birth weight (Akahori et al. 2012 [n=120]; Knopp et al. 1985 [n=272], Laughon et al. 2009 [n=212], Morken et al. 2014 [n=953]), whereas results from smaller studies have been inconsistent (Davison and Hytten 1974 [n=10]; Dunlop et al. 1978 [n=34]; Dunlop 1981 [n=25]; Duvekot et al. 1995 [n=16]; Gibson 1973 [n=21]). Given the new evidence, there is reason to believe a true association exists between maternal GFR during pregnancy and birth weight. Yet, our results, which are based on the association between GFR and birth weight from three studies with individual-specific paired GFR and birth weight measurements (Dunlop 1981, Gibson 1973, Morken et al. 2014), are not in contrast with the conclusion of Lam et al. (2014). Rather than suggesting that GFR is the sole driver of the association between prenatal PFAS and birth weight, our results indicate that a portion of the association may be attributable to confounding

by GFR and that effect estimates may be overpredicted in epidemiologic studies where GFR is not accounted for.

Conclusion

Results from our simulations suggest that epidemiologic studies of prenatal PFAS and birth weight may have overestimated the strength of the association. This study adds to existing studies demonstrating that pharmacokinetic models can be used to provide insight into the direction (Watkins et al. 2013) and the strength of epidemiologic associations (Verner et al. 2013). By combining results from epidemiologic studies with pharmacokinetic analyses, researchers will be able to identify underlying factors that can positively or negatively confound associations and to estimate their contribution to observed effect estimates.

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Table 1. Distributions of parameters used in the Monte Carlo simulations.

Parameter	PFAS	Mean±SD	Min	Max
Standardized glomerular filtration rate (GFR_{ratio})[a]	-	1.000±0.246	0.508	1.492
Residual birth weight (g)[b]	-	0±441	-882	882
Pre-pregnancy body weight (kg)[c]	-	70.3±14.3	37.0	134.0
Volume of liver as a fraction of BW[d]	-	0.026±0.004	0.018	0.034
Liver: plasma partition coefficient[d]	PFOS	3.720±0.558	2.604	4.836
	PFOA	2.200±0.330	1.540	2.860
Rest of body:plasma partition coefficient[d]	PFOS	0.200±0.030	0.140	0.260
	PFOA	0.120±0.018	0.084	0.156
Free fraction in maternal plasma[d]	PFOS	0.025±0.004	0.017	0.033
	PFOA	0.020±0.003	0.014	0.026
Free fraction in fetal plasma[d]	PFOS	0.025±0.004	0.017	0.033
	PFOA	0.020±0.003	0.014	0.026
Resorption maximum velocity ($mg/h/kg^{0.75}$)[d]	PFOS	3.500±0.525	2.450	4.550
	PFOA	10.00±1.50	7.000	13.000
Affinity constant (mg/l)[d]	PFOS	0.023±0.003	0.017	0.029
	PFOA	0.055±0.008	0.039	0.071
Initial plasma PFAS levels (ng/ml)[e]	PFOS	13.02±4.79	0.01	100.00
	PFOA	2.53±1.13	0.01	100.00

[a]Distribution of GFR_{ratio} pooled from the three selected studies (Dunlop 1981; Gibson 1973; Morken et al. 2014). [b]From the GFR_{ratio}-birth weight meta-analytic regression. [c]Distribution of pre-pregnancy body weight from the Norwegian Mothers and Babies Study (MOBA). [d]Mean values taken from Loccisano et al. (2013). SDs were calculated assuming a coefficient of variation of 15% and bounds were set to ± 2SD. [e]Values presented are arithmetic means and SDs.

All distributions were assumed to be normal.

Table 2. Sensitivity analyses evaluating the influence of the PFAS distribution and the strength of the GFR-birth weight association on the simulated change in birth weight (g) per ng/ml increase in PFAS levels attributable to GFR.

Multiplier - Mean PFAS level[a]	Multiplier - coefficient of variation PFAS levels[b]	Multiplier - Beta of the GFR-birth weight association[c]	Sampling seed	Change in birth weight (g) per ng/ml increase in maternal plasma PFAS level at delivery β (95% CI)	Change in birth weight (g) per ng/ml increase in cord plasma PFAS level at delivery β (95% CI)
PFOA					
1 (main results)	1 (main results)	1 (main results)	123456789	-7.92 (-9.42, -6.43)	-7.13 (-8.46, -5.80)
2	1	1	123456789	-3.96 (-4.70, -3.21)	-3.56 (-4.23, -2.90)
0.5	1	1	123456789	-15.88 (-18.86, -12.89)	-14.28 (-16.95, -11.62)
1	2	1	123456789	-3.29 (-4.19, -2.40)	-3.20 (-4.03, -2.37)
1	0.5	1	123456789	-26.07 (-28.75, -23.39)	-17.59 (-19.67, -15.51)
1	1	2	123456789	-13.40 (-16.80, -14.92)	-11.66 (-13.01, -10.31)
1	1	0.5	123456789	-5.17 (-6.66, -3.68)	-4.86 (-6.18, -3.53)
1	1	1	11111	-8.51 (-10.01, -7.02)	-7.33 (-8.67, -5.99)
1	1	1	99999	-7.77 (-9.27, -6.28)	-6.89 (-8.23, -5.56)
PFOS					
1 (main results)	1 (main results)	1 (main results)	123456789	-1.46 (-1.81, -1.11)	-2.72 (-3.40, -2.04)
2	1	1	123456789	-0.73 (-0.91, -0.56)	-1.36 (-1.70, -1.02)
0.5	1	1	123456789	-2.93 (-3.63, -2.23)	-5.45 (-6.81, -4.09)
1	2	1	123456789	-0.54 (-0.75, -0.34)	-1.15 (-1.57, 0.73)
1	0.5	1	123456789	-5.16 (-5.80, -4.51)	-6.60 (-7.65, -5.55)
1	1	2	123456789	-2.77 (-3.12, -2.41)	-5.01 (-5.70, -4.32)
1	1	0.5	123456789	-0.81 (-1.16, -0.46)	-1.57 (-2.25, -0.90)
1	1	1	11111	-1.80 (-2.15, -1.44)	-3.13 (-3.82, -2.45)
1	1	1	99999	-1.42 (-1.77, -1.07)	-2.68 (-3.36, -2.00)

[a]Mean values were 2.53 ng/ml for PFOA and 13.02 ng/ml for PFOS in main analyses. [b]Coefficients of variation were 0.446 for PFOA and 0.368 for PFOS in main analyses. [c]The Beta in of the GFR-birth weight association was 175.5 g per 1 unit increase GFR_{ratio} in the main analyses.

Figure Legends

Figure 1. Structure of human gestation PBPK model for PFOS and PFOA adapted from Loccisano et al. (2013).

Figure 2. Comparison of simulated vs. measured levels from Glynn et al. (2012) and Monroy et al. (2008). Distributions of simulated levels are from 10,000 Monte Carlo simulations.

Figures 3A and B. Difference in birth weight (g) per 1 ng/ml increase in reported and simulated PFOA (A) and PFOS (B) levels. In the Reported section, the size of the square represents the weight of each study in the calculation of the overall meta-analytic association. The heterogeneity chi-square for the PFOA meta-analysis was 7.4 (not statistically significant), and for PFOS was 20.1 ($p<0.05$), both with 6 df. The summary beta coefficient for PFOS was from a random effects model.

Figure 1.

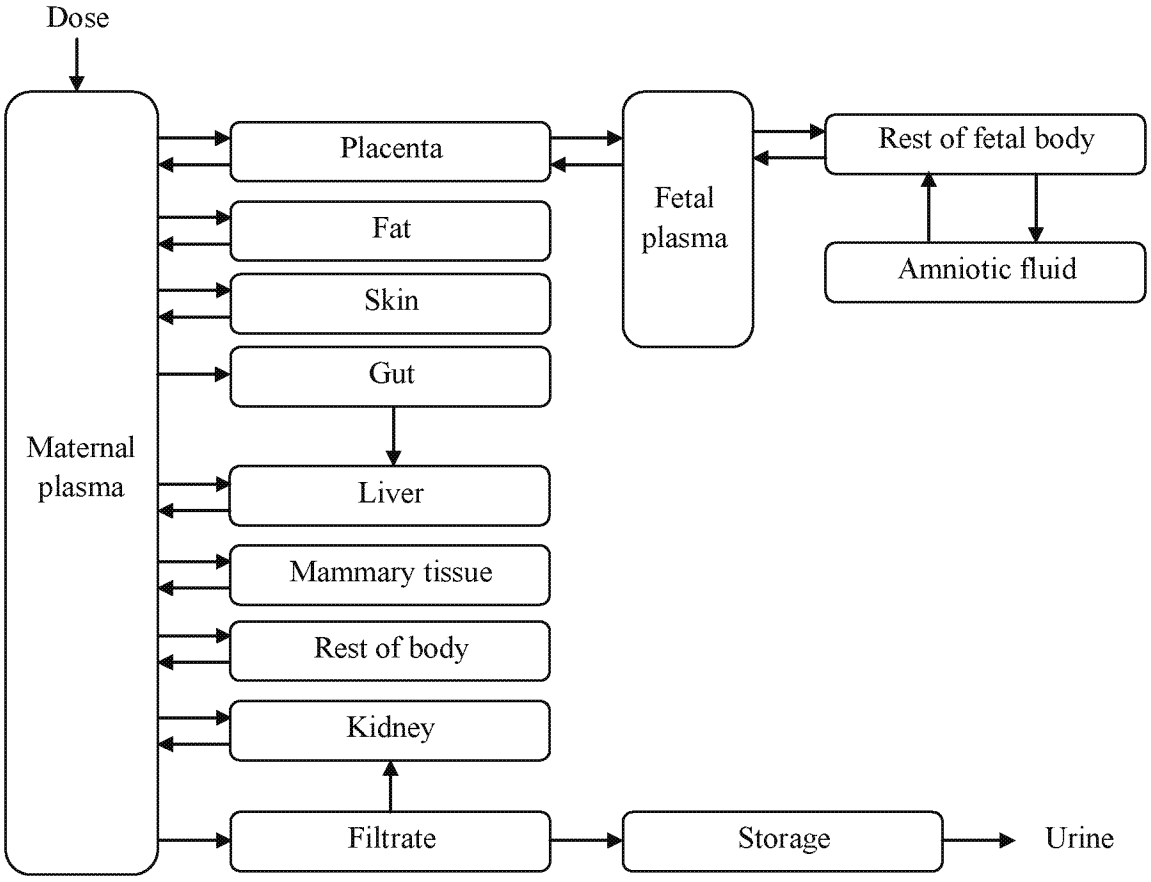


Figure 2.

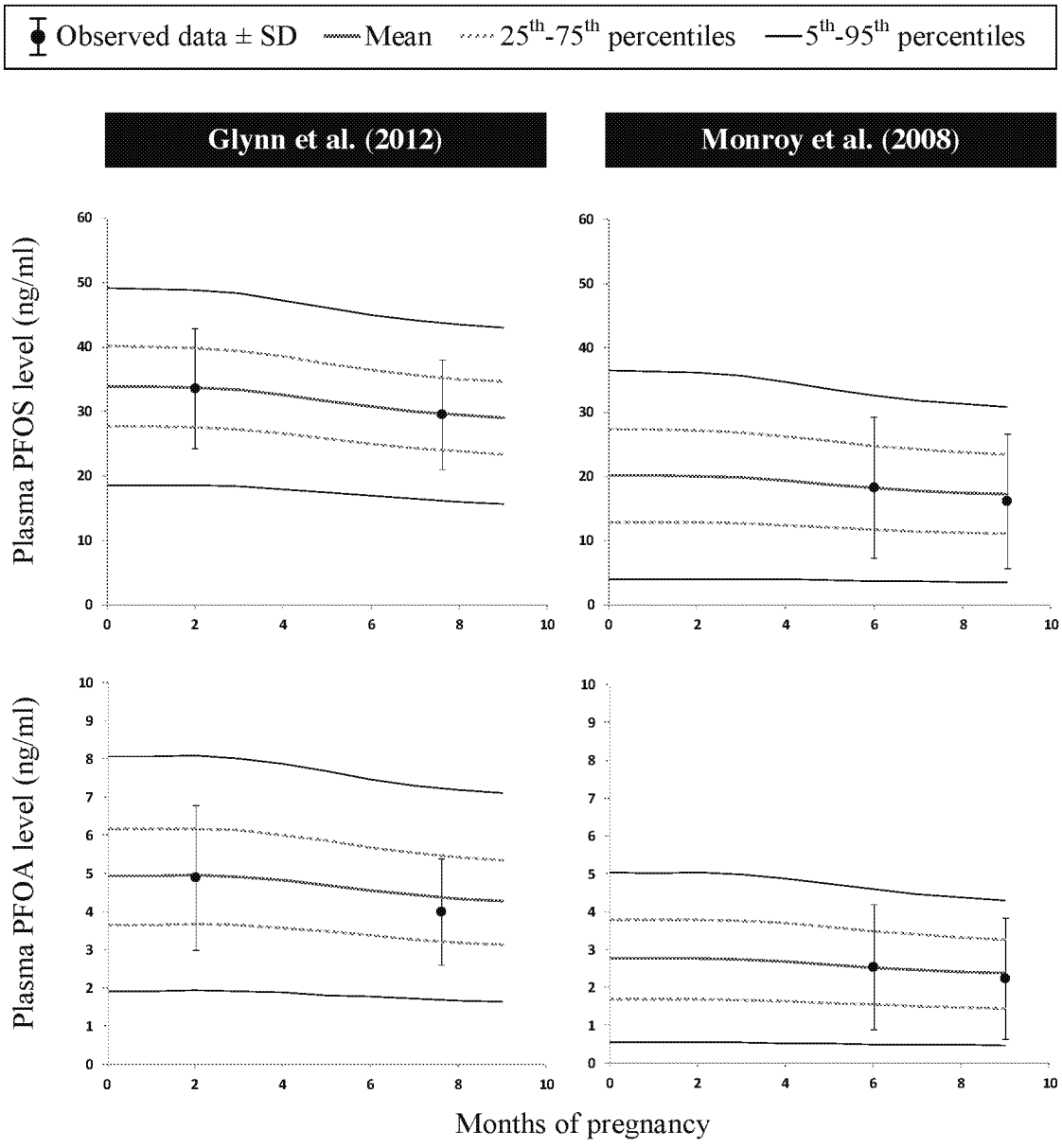

Observed data ± SD
Mean
25th-75th percentiles
5th-95th percentiles
Glynn et al. (2012)
Monroy et al. (2008)
Plasma PFOS level (ng/ml)
Plasma PFOA level (ng/ml)
Months of pregnancy

Figure 3A.

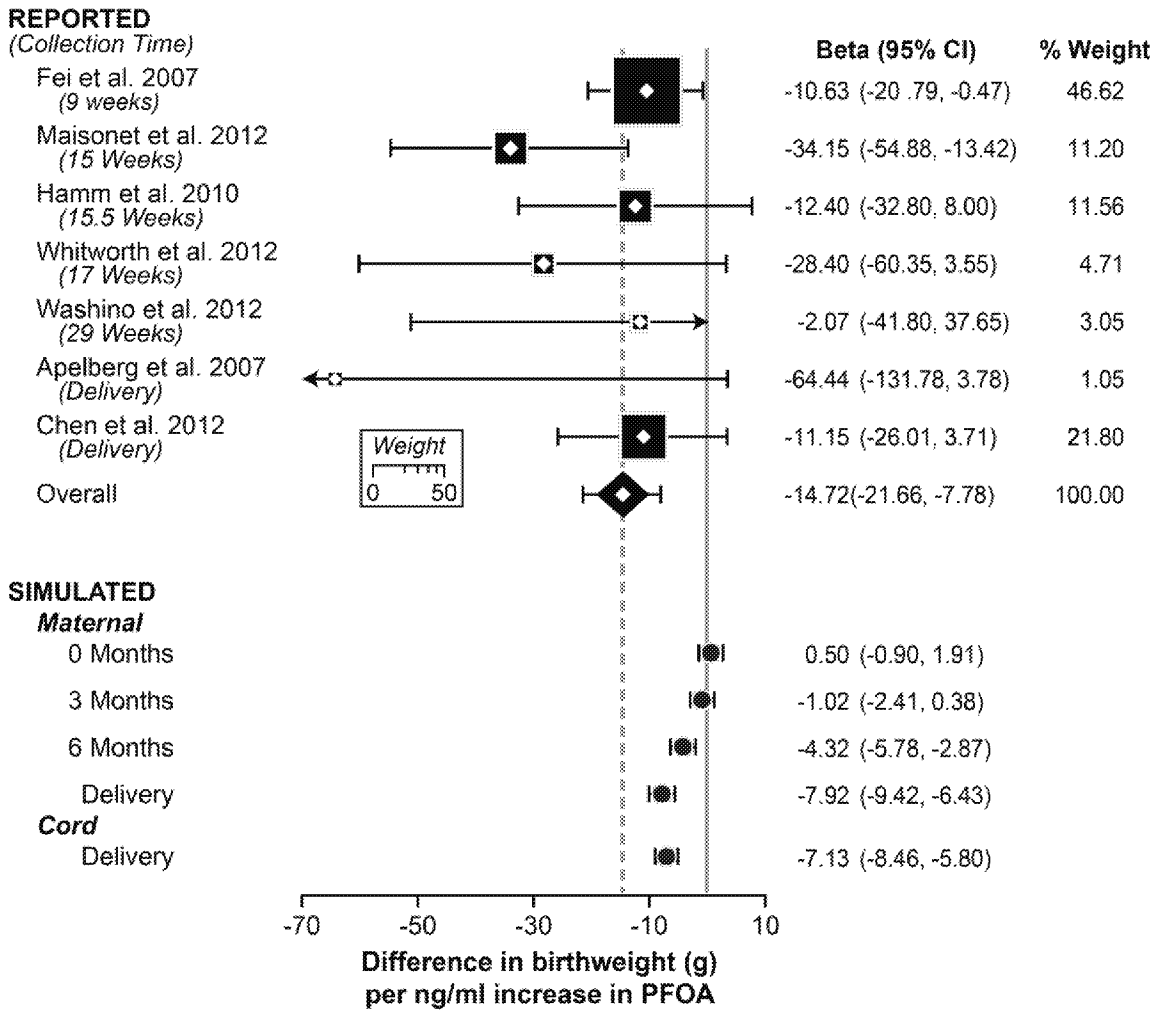
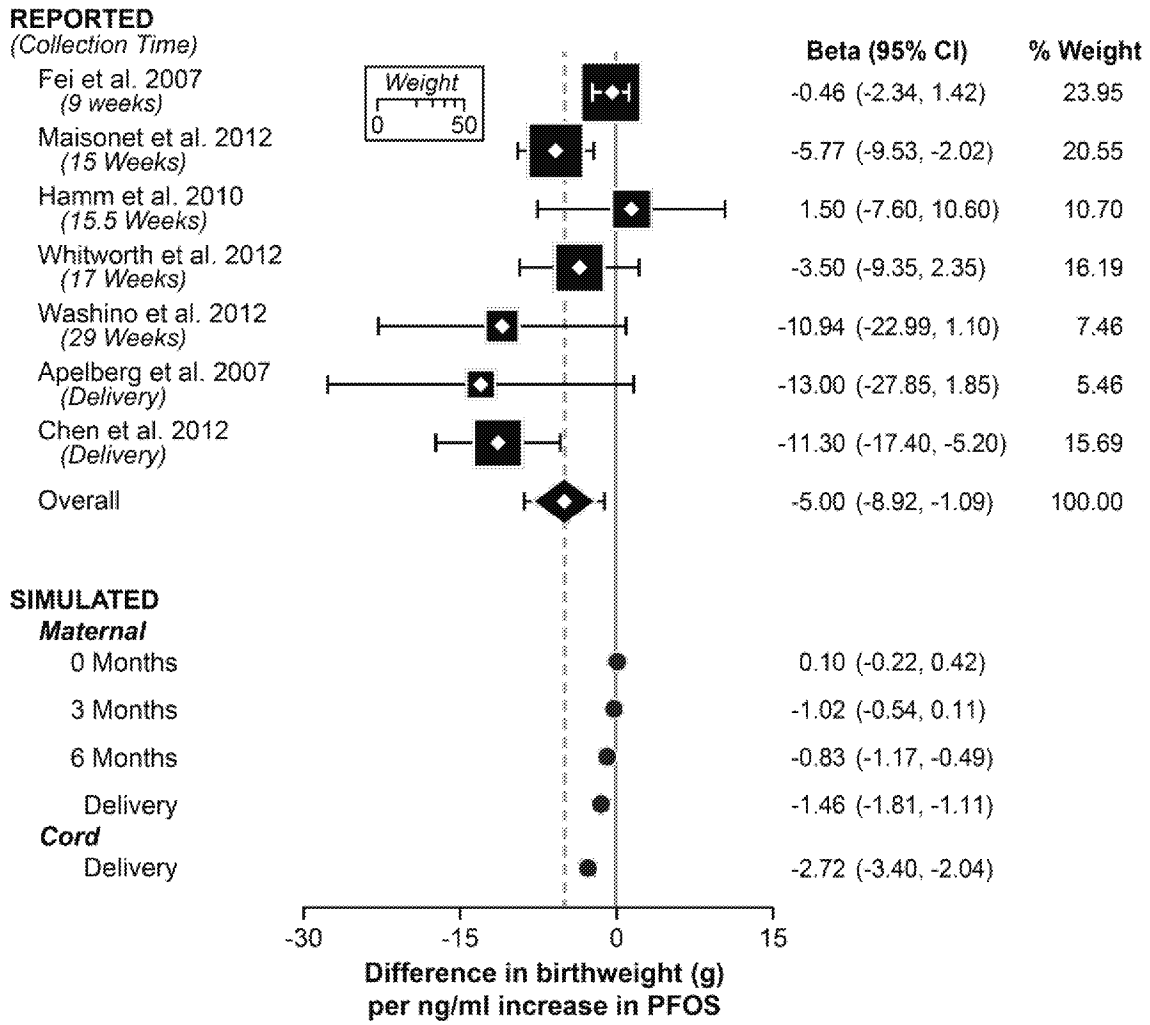


Figure 3B.



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Supplemental Material

Associations of Perfluoroalkyl Substances (PFASs) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK)

Marc-André Verner, Anne E. Loccisano, Nils-Halvdan Morken, Miyoung Yoon, Huali Wu, Robin McDougall, Mildred Maisonet, Michele Marcus, Reiko Kishi, Chihiro Miyashita, Mei-Huei Chen, Wu-Shiun Hsieh, Melvin E. Andersen, Harvey J. Clewell III, and Matthew P. Longnecker

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Table S2. Sensitivity coefficients for the most sensitive model parameters.
PBPK Model Code
References

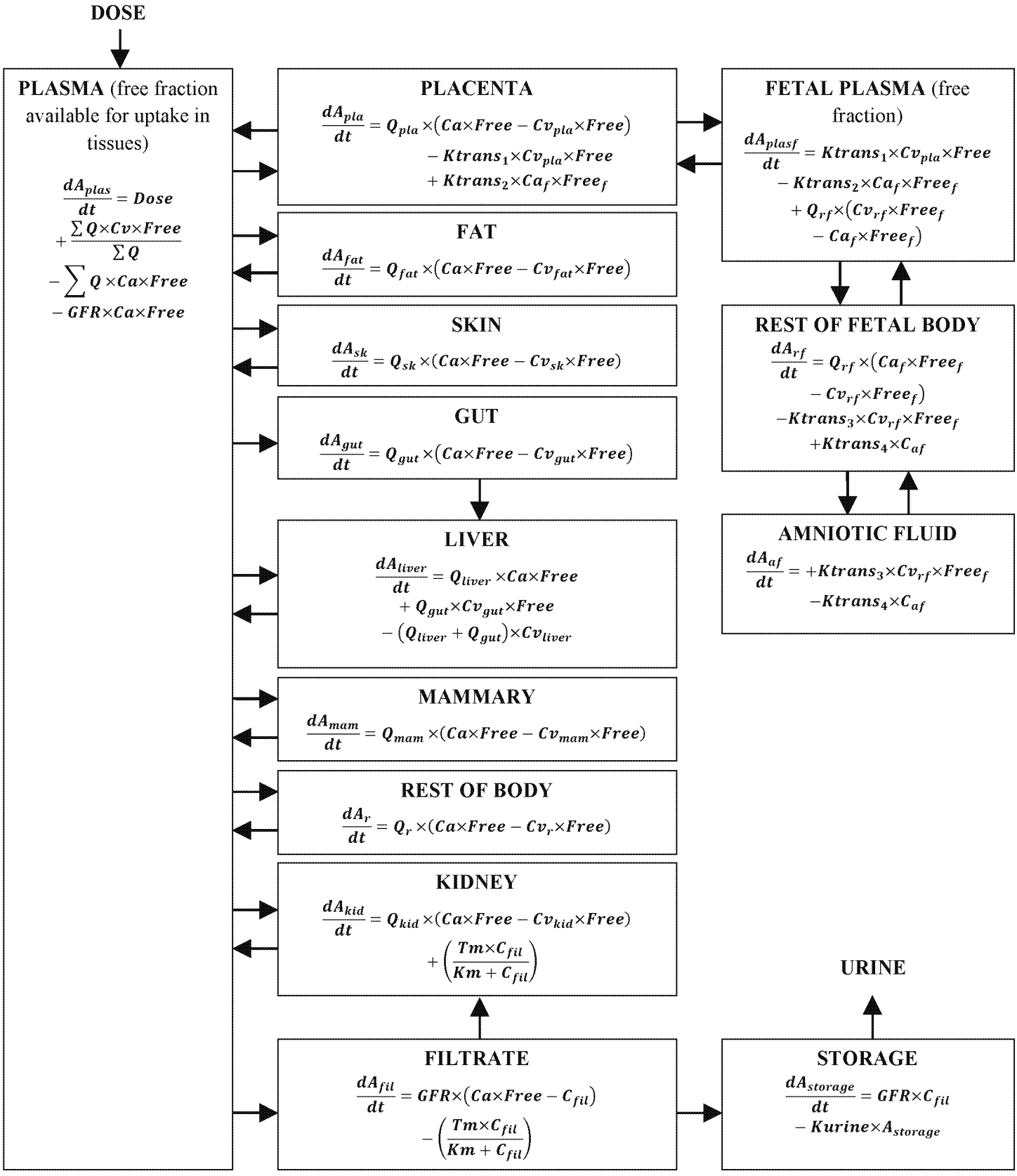

Figure S1. Conceptual representation of the PBPK model (Loccisano et al. 2013) including mass-balance differential equations. The different symbols are spelled out on next page.

Symbols

A_{af}	Amount in amniotic fluid
A_{fat}	Amount in fat
A_{fil}	Amount in filtrate
A_{gut}	Amount in gut
A_{kid}	Amount in kidneys
A_{liver}	Amount in liver
A_{mam}	Amount in mammary tissue
A_{pla}	Amount in placenta
A_{plas}	Amount in plasma (mother)
A_{plasf}	Amount in plasma (fetus)
A_r	Amount in rest of body (mother)
A_{rf}	Amount in rest of body (fetus)
A_{sk}	Amount in skin
$A_{storage}$	Amount in storage compartment
Ca	Arterial plasma concentration (mother)
Ca_f	Arterial plasma concentration (foetus)
C_{af}	Concentration in amniotic fluid
C_{fil}	Concentration in filtrate
Cv	Concentration in total venous plasma
Cv_{fat}	Concentration in venous plasma from the fat
Cv_{gut}	Concentration in venous plasma from the gut
Cv_{kid}	Concentration in venous plasma from the kidneys
Cv_{liver}	Concentration in venous plasma from the liver
Cv_{mam}	Concentration in venous plasma from the mammary tissue
Cv_{pla}	Concentration in venous plasma from the placenta
Cv_r	Concentration in venous plasma from the rest of body (mother)
Cv_{rf}	Concentration in venous plasma from the rest of body (fetus)
Cv_{sk}	Concentration in venous plasma from skin
Free	Free fraction in plasma (mother)
$Free_f$	Free fraction in plasma (fetus)
GFR	Glomerular filtration rate
Km	Renal transporter affinity constant
Ktrans1	Placental diffusion constant (from mother to fetus)
Ktrans2	Placental diffusion constant (from fetus to mother)
Ktrans3	Transfer constant (from fetus to amniotic fluid)
Ktrans4	Transfer constant (from amniotic fluid to fetus)
Kurine	Urinary elimination rate constant
Q	Plasma perfusion rate
Q_{fat}	Plasma perfusion rate to the fat
Q_{gut}	Plasma perfusion rate to the gut
Q_{kid}	Plasma perfusion rate to the kidneys
Q_{liver}	Plasma perfusion rate to the liver
Q_{mam}	Plasma perfusion rate to the mammary tissue
Q_{pla}	Plasma perfusion rate to the placenta
Q_r	Plasma perfusion rate to the rest of body (mother)
Q_{rf}	Plasma perfusion rate to the rest of body (fetus)
Q_{sk}	Plasma perfusion rate to the skin
Tm	Renal transporter maximum resorption velocity

Table S1. List of studies included or excluded from meta-analysis of birth weight and PFAS, with a brief description of each.

Source	Country	Period	N	Comments
Included				
Apelberg et al. 2007	U.S.A.	2004-2005	293	
Fei et al. 2007	Denmark	1996-2002	1,399	
Hamm et al. 2009	Canada	2005-2006	252	
Washino et al. 2009	Japan	2002-2005	428	
Chen et al. 2012	Taiwan	2004-2005	429	
Whitworth et al. 2012	Norway	2003-2004	849	
Maisonet et al. 2012	Great Britain	1991-1992	422	
Excluded				
Inoue et al. 2004	Japan	2003	15	Univariate. Birth weight was the independent variable.
Monroy et al. 2008	Canada	2004-2005	89	Univariate. Birth weight was the independent variable.
Nolan, 2009	U.S.A.	2003-2005	1,555	Univariate. Exposure was based on water district.
Stein et al. 2009	U.S.A.	2005-2006	1,589-4,561	PFAS measured after pregnancy. Low birth weight was the outcome.
Kim et al. 2011	Korea	2008-2009	44	Regression coefficient not presented.
Savitz et al. 2012a	U.S.A.	2005-2006	11,737	PFAS estimated from model. Low birth weight was the outcome.
Savitz et al. 2012b	U.S.A.	1990-2004	8,253	PFAS estimated from model.
Halldorrson et al. 2012	Denmark	1988-1989	655	Univariate
Wu et al. 2012	China	2007	158	See footnote

We conducted in the Pubmed search in 2012 and after the manuscript for this article was provisionally accepted we repeated the search as a check. The search in April of 2015 revealed that the Wu et al. (2012) article also met the inclusion criterion, and had data on PFOA. When we repeated the meta-analysis for PFOA including the Wu et al. (2012) results, the summary coefficient from a random effects models was -12.4 g birth weight (SE 3.7), which was close to our original results (-14.7 g). To calculate this estimate, however, we assumed that a $\log_{10}$ increase in PFOA was equivalent to 50 ng/ml, and we were not confident about this re-expression. Thus, we did not update the meta-analysis. We also repeated the meta-regression for PFOA, and with the data from Wu et al. (2012) included, the coefficient for week of blood draw was not statistically significant, as before.

Table S2. Sensitivity coefficients for the most sensitive model parameters.

	Initial body weight	Liver volume as a fraction of body weight	Liver: plasma partition coefficient	Rest of body:plasma partition coefficient	Free fraction in maternal plasma	Free fraction in fetal plasma	Resorption maximum velocity	Affinity constant	Standardized glomerular filtration rate (GFR_{ratio})
PFOS									
Maternal plasma									
1st month	0.00025482	0.00004976	0.00004027	0.00004649	0.00013986	0.00000000	0.00010732	0.00013918	0.00027270
2nd month	0.00055318	0.00010385	0.00008401	0.00009799	0.00030334	0.00000027	0.00023395	0.00029977	0.00057214
3rd month	0.00087194	0.00017493	0.00014362	0.00017316	0.00047710	0.00000274	0.00036859	0.00046620	0.00089098
4th month	0.00125927	0.00027143	0.00022592	0.00028111	0.00068930	0.00001233	0.00053090	0.00066052	0.00126904
5th month	0.00172690	0.00039098	0.00032767	0.00041777	0.00095053	0.00003680	0.00072525	0.00088372	0.00171160
6th month	0.00210177	0.00048521	0.00040702	0.00052628	0.00116418	0.00006802	0.00088081	0.00105471	0.00205999
7th month	0.00242076	0.00056382	0.00047244	0.00061720	0.00134951	0.00010523	0.00101352	0.00119526	0.00235614
8th month	0.00268585	0.00062637	0.00052392	0.00069034	0.00150833	0.00015136	0.00112232	0.00130701	0.00259492
Delivery	0.00292021	0.00068096	0.00056859	0.00075549	0.00165564	0.00021251	0.00121457	0.00139978	0.00279755
Cord plasma									
Delivery	0.00188033	0.00032544	0.00063431	0.00051174	0.00269457	0.00457312	0.00078186	0.00095558	0.00249216
PFOA									
Maternal plasma									
1st month	0.00008960	0.00002928	0.00002016	0.00002822	0.00006194	0.00000000	0.00004355	0.00004470	0.00010143
2nd month	0.00019174	0.00005798	0.00004023	0.00005571	0.00012973	0.00000007	0.00009355	0.00009600	0.00020815
3rd month	0.00029740	0.00008806	0.00006425	0.00008698	0.00019617	0.00000068	0.00014487	0.00014854	0.00031847
4th month	0.00042126	0.00012149	0.00009465	0.00012450	0.00026942	0.00000292	0.00020422	0.00020918	0.00044361
5th month	0.00056227	0.00015499	0.00013065	0.00016572	0.00034664	0.00000811	0.00027093	0.00027728	0.00058004
6th month	0.00066513	0.00017537	0.00015760	0.00019371	0.00039773	0.00001411	0.00031994	0.00032729	0.00067768
7th month	0.00074250	0.00018789	0.00017846	0.00021318	0.00043214	0.00002059	0.00035806	0.00036608	0.00075302
8th month	0.00079924	0.00019460	0.00019334	0.00022552	0.00045529	0.00002840	0.00038728	0.00039585	0.00080887
Delivery	0.00084311	0.00019813	0.00020495	0.00023402	0.00047216	0.00003864	0.00041043	0.00041948	0.00085270
Cord plasma									
Delivery	0.00091908	0.00026732	0.00032616	0.00026743	0.00115986	0.00147319	0.00056451	0.00044270	0.00111788

Sensitivity coefficients were calculated by adapting the M code of the Morris Test included in the acslX Optimum suite of tools (Aegis Technologies Inc., Huntsville, AL, USA) to our study.

```
! MODEL CODE FOR ACSLX (AEGIS TECHNOLOGIES GROUP, INC, HUNSTVILLE, AL, USA)

PROGRAM PFAS_GESTATION_MC

!-----------------------------------------------!
! PBPK model for PFOS/PFOA in pregnant human    !
! Coded by Anne Loccisano                       !
! Modified by Marc-Andre Verner                 !
!                                               !
! Units                                         !
! Amounts: mg                                   !
! Volumes: L                                    !
! Time: h                                       !
!-----------------------------------------------!

INITIAL

!-------------------------------------------------------------------------------------------------
! Compound (Only one compound can have a value of "1")
constant PFOS = 1     ! PFOS? (1:Yes, 0:No)
constant PFOA = 0     ! PFOA? (1:Yes, 0:No)

!-------------------------------------------------------------------------------------------------
! Initial venous blood concentration (mg/l or ug/ml)
constant CVINIT = 0.013

!-------------------------------------------------------------------------------------------------
! Simulation time in hours (9 months = 6570 h)
constant TSTOP = 6570

!-------------------------------------------------------------------------------------------------
! Constant physiological parameters

! Body weight
constant BWINIT = 60.9        ! Pre-pregnancy body weight (kg)

! Maternal blood flows
constant QCC    = 20.0        ! Cardiac blood output (L/h/kg**0.75)
constant QLC    = 0.065       ! Fraction cardiac output going to liver through hepatic artery
constant QSkC   = 0.058       ! Fraction of cardiac output going to skin
constant QGC    = 0.181       ! Fraction of cardiac output going to gut
constant QMamC  = 0.027       ! Initial fraction of cardiac output going to mammary tissue
constant QFatC  = 0.052       ! Initial fraction of cardiac output going to fat
```

```
TABLE DQK,1,26/0,8,10,11,12,13,15,17,18,19,22,23,24,25,26,27,28,29,31,32,33,34,36,37,38,40, &
24.42,50.58,45.42,51.36,57.12,46.32,44.73,45.36,41.8,46.53,46.08,47.85,43.05,41.16,47.52,43.41,&
48.57,46.36,36.68,39.44,46.46,44.13,26.7,33.92,35.46,40.26/  ! Renal plasma flow (L/h) [INDEX:WK]
TABLE DQFil,1,40/0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28, &
29,30,31,32,33,34,35,36,37,38,39,6.0,6.0,6.0,6.0,6.0,6.03,6.09,6.18,6.32,6.50,6.72,6.97,7.23, &
7.50,7.76,8.01,8.23,8.43,8.60,8.75,8.86,8.95,9.01,9.05,9.06,9.06,9.04,8.99,8.95,8.88,8.81,8.72, &
8.62,8.52,8.41,8.29,8.16,8.03,7.89,7.75/   ! Glomerular filtration rate (L/h) [INDEX:WK]

! Maternal tissue volumes
constant VLC      = 0.026    ! Liver volume as a fraction of initial BW
constant VKC      = 0.004    ! Kidney volume as a fraction of initial BW
constant VfilC    = 0.0004   ! Filtrate compartment volume as a fraction of initial BW
constant VGC      = 0.0171   ! Gut volume as a fraction of initial BW
constant VMamC    = 0.0062   ! Mammary tissue volume as a fraction of initial BW
constant VFatC    = 0.214    ! Fat volume as a fraction of initial BW
constant VSk      = 0.0972   ! Skin volume (L)
TABLE DVPlasC,1,28/9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,&
33,34,35,36,0.0442,0.0445,0.0448,0.0451,0.0454,0.0456,0.0458,0.0460,0.0461,0.0462,0.0463, &
0.0464,0.0464,0.0464,0.0464,0.0463,0.0463,0.0461,0.0460,0.0458,0.0456,0.0454,0.0451,0.0448, &
0.0445,0.0441,0.0437,0.0433/     ! Maternal plasma volume as a fraction of BW [INDEX:WK]
TABLE DHtc,1,9/0,11,16,20,24,28,32,36,40,0.38,0.372,0.356,0.354,0.349,0.347, &
0.346,0.354,0.367/     ! Hematocrit [INDEX:WK]
TABLE VAFX,1,20/0,8,9,10,11,12,16,18,20,22,24,26,28,30,32,34,36,38,40,42,0.0237,0.0235,&
0.0234,0.0232,0.0766,0.124,0.195,0.284,0.361,0.646,0.640,0.687,0.770,0.824,0.776,0.817, &
0.817,0.799,0.530,0.506/     ! Amniotic fluid volume (L) [INDEX:WK]

!-----------------------------------------------------------------------------------------
! Chemical specific parameters

! Tissue:plasma partition coefficients (from rat)
constant PFOS_PL   = 3.72     ! PFOS Liver:plasma partition coefficient
constant PFOS_PFat = 0.14     ! PFOS Fat:plasma partition coefficient
constant PFOS_PK   = 0.80     ! PFOS Kidney:plasma partition coefficient
constant PFOS_PSk  = 0.29     ! PFOS Skin:plasma partition coefficient
constant PFOS_PR   = 0.20     ! PFOS Rest of body:plasma partition coefficient
constant PFOS_PG   = 0.57     ! PFOS Gut:plasma partition coefficient
constant PFOS_PMam = 0.16     ! PFOS Mammary tissue:plasma partition coefficient
constant PFOS_PPla = 0.41     ! PFOS Placenta:plasma partition coefficient
constant PFOS_PRF  = 0.20     ! PFOS Rest of fetal body:plasma partition coefficient
constant PFOA_PL   = 2.2      ! PFOA Liver:plasma partition coefficient
constant PFOA_PFat = 0.04     ! PFOA Fat:plasma partition coefficient
constant PFOA_PK   = 1.05     ! PFOA Kidney:plasma partition coefficient
constant PFOA_PSk  = 0.1      ! PFOA Skin:plasma partition coefficient
```

```
constant PFOA_PR   = 0.12     ! PFOA Rest of body:plasma partition coefficient
constant PFOA_PG   = 0.05     ! PFOA Gut:plasma partition coefficient
constant PFOA_PMam = 0.13     ! PFOA Mammary tissue:plasma partition coefficient
constant PFOA_PPla = 0.28     ! PFOA Placenta:plasma partition coefficient
constant PFOA_PRF  = 0.12     ! PFOA Rest of fetal body:plasma partition coefficient

PL   = PFOS*PFOS_PL   + PFOA*PFOA_PL     ! Liver:plasma partition coefficient
PFat = PFOS*PFOS_PFat + PFOA*PFOA_PFat   ! Fat:plasma partition coefficient
PK   = PFOS*PFOS_PK   + PFOA*PFOA_PK     ! Kidney:plasma partition coefficient
PSk  = PFOS*PFOS_PSk  + PFOA*PFOA_PSk    ! Skin:plasma partition coefficient
PR   = PFOS*PFOS_PR   + PFOA*PFOA_PR     ! Rest of the body:plasma partition coefficient
PG   = PFOS*PFOS_PG   + PFOA*PFOA_PG     ! Gut:plasma partition coefficient
PMam = PFOS*PFOS_PMam + PFOA*PFOA_PMam   ! Mammary:plasma partition coefficient
PPla = PFOS*PFOS_PPla + PFOA*PFOA_PPla   ! Placenta:plasma partition coefficient
PRF  = PFOS*PFOS_PRF  + PFOA*PFOA_PRF    ! Rest of fetal body:plasma partition coefficient

! Renal reabsorption
constant PFOS_TMC = 3.5                  ! Reabsorption maximum PFOS (mg/h/kg**0.75)
constant PFOA_TMC = 10.0                 ! Reabsorption maximum PFOA (mg/h/kg**0.75)
TMC = PFOS*PFOS_TMC + PFOA*PFOA_TMC      ! Reabsorption maximum (mg/h/kg**0.75)
constant PFOS_KT = 0.023                 ! Affinity constant PFOS (mg/L)
constant PFOA_KT = 0.055                 ! Affinity constant PFOA (mg/L)
KT  = PFOS*PFOS_KT + PFOA*PFOA_KT        ! Affinity constant (mg/L)

! Binding constants
constant PFOS_Free  = 0.025              ! Free fraction of PFOS in maternal plasma
constant PFOA_Free  = 0.020              ! Free fraction of PFOA in maternal plasma
Free  = PFOS*PFOS_Free+PFOA*PFOA_Free    ! Free fraction in maternal plasma
constant PFOS_FreeF = 0.025              ! Free fraction in fetal plasma PFOS
constant PFOA_FreeF = 0.020              ! Free fraction in fetal plasma PFOA
FreeF = PFOS*PFOS_FreeF+PFOA*PFOA_FreeF  ! Free fraction in fetal plasma

! Urinary excretion
constant kurinec = 0.005      ! Urinary elimination rate constant (/h/kg**-0.25)

! Placental transfer
constant PFOS_k1c = 0.46                   ! Mom to fetus PFOS (L/h/kg**0.75)
constant PFOA_k1c = 0.46                   ! Mom to fetus PFOA (L/h/kg**0.75)
ktrans1c = PFOS*PFOS_k1c + PFOA*PFOA_k1c   ! Mom to fetus (L/h/kg**0.75)
constant PFOS_k2c = 1.01                   ! Fetus to mom PFOS (L/h/kg**0.75)
constant PFOA_k2c = 0.46                   ! Fetus to mom PFOA (L/h/kg**0.75)
ktrans2c = PFOS*PFOS_k2c + PFOA*PFOA_k2c   ! Fetus to mom (L/h/kg**0.75)
```

```
! Amniotic fluid transfer
constant PFOS_k3c = 0.006          ! Amniotic fluid to fetus PFOS (L/h/kg**0.75)
constant PFOA_k3c = 0.008          ! Amniotic fluid to fetus PFOA (L/h/kg**0.75)
ktrans3c = PFOS*0.006+ PFOA*0.008  ! Amniotic fluid to fetus (L/h/kg**0.75)
constant PFOS_k4c = 0.001          ! Fetus to fluid PFOS (L/h/kg**0.75)
constant PFOA_k4c = 0.001          ! Fetus to fluid PFOA (L/h/kg**0.75)
ktrans4c = PFOS*0.001+PFOA*0.001   ! Fetus to fluid (L/h/kg**0.75)

END ! INITIAL

DYNAMIC ! Start dynamic section

ALGORITHM IALG = 15        ! Use CVODE algorithm
CINTERVAL CINT = 100       ! Communication interval
MINTERVAL MINT = 10e-09    ! Minimum interval
MAXTERVAL MAXT = 1.0       ! Maximum interval

DERIVATIVE

!-----------------------------------------------------------------------------------------------
! Timing parameters
GD = T/24     ! Gestation day
WK = GD/7     ! Gestation week

!-----------------------------------------------------------------------------------------------
! Organ volume calculation

! Fetal growth
VFet_av = (3.779*exp(-16.081*(exp(-5.67e-4*(GD*24)))) &
         + 3.833*exp(-140.178*(exp(-7.01e-4*(GD*24)))))  ! Average fetal volume (L)
constant Birthweight = 3.50857                           ! Birth weight (kg)
VFet                 = (Birthweight/3.50857)*VFet_av     ! Adjustment of fetal growth (L)

! Amniotic fluid
constant AF_VAF = 1.0      ! Adjustment factor for amniotic fluid
VAF = AF_VAF*VAFX(WK)      ! Amniotic fluid volume (L)

! Hematocrit
constant HtcF   = 0.5     ! Fetal hematocrit
constant AF_Htc = 1.0     ! Adjustment factor for maternal hematocrit
HtcINIT = AF_Htc*DHtc(0)  ! Initial maternal hematocrit
Htc     = AF_Htc*DHtc(WK) ! Maternal hematocrit
```

```
! Plasma volumes
constant VPlasFC   = 0.0428             ! Fetal plasma volume as a fraction of fetal weight
constant AF_VPlasF = 1.0                ! Adjustment factor for fetal plasma volume
VPlasF    = AF_VPlasF*VPlasFC*VFet      ! Fetal plasma volume (L)
constant AF_VPlas = 1.0                 ! Adjustment factor for maternal plasma volume
VPlasINIT = AF_VPlas*DVPlasC(0)*BWINIT  ! Initial plasma volume (L)
VPlas     = AF_VPlas*DVPlasC(WK)*BW     ! Maternal plasma volume (L)

! Placenta volume
constant AF_VPla = 1.0                                  ! Adjustment factor for placenta volume
VPla_av  = (0.85*exp(-9.434*(exp(-5.23e-4*(GD*24))))) ! Average placenta volume (L)
Vplainit = AF_VPla*0.85*exp(-9.434*(exp(0)))            ! Initial placenta volume (L)
VPla     = AF_VPla*VPla_av                              ! Placenta volume (L)

! Other tissue volumes
VL     = VLC*BWINIT                       ! Liver volume (L)
VK     = VKC*BWINIT                       ! Kidney volume (L)
Vfil   = VfilC*BWINIT                     ! Fitrate compartment volume (L)
VG     = VGC*BWINIT                       ! Gut volume (L)
VMamINIT = VMamC*BWINIT                   ! Initial mammary volume (L)
VFatINIT = VFatC*BWINIT                   ! Initial fat volume (L)
VR     = (0.84*BWINIT)-VL-VK-VFil &
         -VG-VPlasINIT-VSk-VMamINIT &
         -VFatINIT                        ! Rest of maternal body volume (L)
VRF    = (0.92*VFet)-VPlasF               ! Volume of rest of fetal body (L)

VMam   = BWINIT*(((VMamC+(0.0065*exp(-7.444868477* &
         (exp(-0.000678*(GD*24))))))))    ! Mammary tissue volume (L)
VFat   = BWINIT*(((VFatC+(0.09*exp(-12.90995862* &
         (exp(-0.000797*(GD*24))))))))    ! Fat volume (L)
BW     = BWINIT+(VFat-VFATINIT)+(VMam-VMamINIT) &
         +VPla+VFet+VAF                   ! Body weight (kg)

!-------------------------------------------------------------------------------------------
! Blood flow calculation

! Fetal cardiac output
constant QFetC      = 24.0      ! Fetal cardiac blood output (L/h/kg fetal BW)
QFet = QFetC*VFet*(1-HtcF)      ! Fetal cardiac plasma output using fetal hematocrit (L/h)
QRF  = QFet                     ! Flow to rest of body (L/h)

! Other flows
QCINIT   = QCC*BWINIT**0.75                     ! Initial cardiac blood output (L/h)
```

```
QCPINIT  = QCINIT*(1-HtcINIT)                              ! Initial cardiac plasma output (L/h)
QL       = QLC*QCPINIT                                     ! Plasma flow to liver (L/h)
QG       = QGC*QCPINIT                                     ! Plasma flow to gut (L/h)
QSk      = QSkC*QCPINIT                                    ! Plasma flow to skin (L/h)
QFatINIT = QFatC*QCPINIT                                   ! Initial flow to fat
QFat     = QFatINIT*(VFat/VFatINIT)                        ! Plasma flow to fat (L/h)
QMamINIT = QMamC*QCPinit                                   ! Intial flow to mammary tissue
QMam     = QMamINIT*(VMam/VMamINIT)                        ! Plasma flow to mammary tissue (L/h)
QC       = QCPINIT+(QFat-QFatINIT)+(QMam-QMamINIT) &
          +(QK-QKINIT)+ (QFil-QFilINIT)+ QPla              ! Total cardiac plasma output (L/h)
QR       = QC-(QL+QK+QG+QSk+QFat+QMam+QPla)                ! Plasma flow to rest of body (L/h)
Qbal     = QC-(QL+QK+QG+QSk+QFat+QMam+QPla+QR)             ! Balance check
kurine   = kurinec*(BWINIT**(-0.25))                       ! Urine elimination from urine storage
Tm       = Tmc*BW**0.75                                    ! Transporter maximum

! Plasma flow to placenta
constant QPlaC = 0.33       ! Plasma flow to placenta as a fraction of fetal cardiac output
QPla = QPlaC*QFet           ! Plasma flow to placenta (L/h)

! Plasma flow to kidney
constant AF_QK = 1.0        ! Adjustment factor for plasma flow to kidney
QKINIT  = AF_QK*DQK(0)      ! Initial plasma flow to kidney
QK      = AF_QK*DQK(WK)     ! Plasma flow to kidney (l/h)

! Glomerular filtration rate
constant Ratio_GFR    = 1.0         ! Adjustment factor for GFR
QFil_R   = DQFil(WK)                ! Average GFR (L/h)
QFilINIT = Ratio_GFR*DQFil(0)       ! Initial GFR (L/h)
QFil     = Ratio_GFR*QFil_R         ! GFR (L/h)

! Placental & amniotic fluid transfer
ktrans1 = ktrans1c*(VFet**0.75)     ! Transfer from placenta to fetal plasma (L/h)
ktrans2 = ktrans2c*(VFet**0.75)     ! Transfer from fetal plasma to placenta (L/h)
ktrans3 = ktrans3c*(VFet**0.75)     ! Transfer from rest of fetal body to amniotic fluid (L/h)
ktrans4 = ktrans4c*(VFet**0.75)     ! Transfer from amniotic fluid to rest of fetal body (L/h)
```

```
!-------------------------------------------------------------------------------
! Sampling times (collection of blood levels)
constant First_sample_t   = 2500    ! Timing of first sample (h)
constant Second_sample_t  = 6569    ! Timing of second sample (h)

! Setting initial values for blood concentrations
! *Flag values 99999 are replaced by simulated levels at recording times
initial
CV_SAMPLE1   = 99999    ! Blood level in 1st sample before it is recorded
CV_SAMPLE2   = 99999    ! Blood level in 2nd sample before it is recorded
CV_SAMPLE_M1 = 99999    ! Blood level at 1st month of pregnancy before it is recorded
CV_SAMPLE_M2 = 99999    ! Blood level at 2nd month of pregnancy before it is recorded
CV_SAMPLE_M3 = 99999    ! Blood level at 3rd month of pregnancy before it is recorded
CV_SAMPLE_M4 = 99999    ! Blood level at 4th month of pregnancy before it is recorded
CV_SAMPLE_M5 = 99999    ! Blood level at 5th month of pregnancy before it is recorded
CV_SAMPLE_M6 = 99999    ! Blood level at 6th month of pregnancy before it is recorded
CV_SAMPLE_M7 = 99999    ! Blood level at 7th month of pregnancy before it is recorded
CV_SAMPLE_M8 = 99999    ! Blood level at 8th month of pregnancy before it is recorded
CV_SAMPLE_M9 = 99999    ! Blood level at 9th month of pregnancy before it is recorded
CORD_SAMPLE  = 99999    ! Cord blood level before it is recorded
end

! Record blood concentration at sampling times
schedule blood_samp1 .xn. (First_sample_t-t)  ! At 1st sampling time, blood_samp1 is "true"
discrete blood_samp1                          ! When blood_samp1 is "true"...
CV_SAMPLE1 = Ca                               ! Record total maternal plasma level
end
schedule blood_samp2 .xn. (Second_sample_t-t) ! At 2nd sampling time, blood_samp2 is "true"
discrete blood_samp2                          ! When blood_samp2 is "true"...
CV_SAMPLE2  = Ca                              ! Record total maternal plasma level
end
schedule blood_samp_m1 .xn. (730-t)           ! At 1st month of pregnancy, blood_samp_m1 is "true"
discrete blood_samp_m1                        ! When blood_samp_m1 is "true"...
CV_SAMPLE_M1  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m2 .xn. (1460-t)          ! At 2nd month of pregnancy, blood_samp_m2 is "true"
discrete blood_samp_m2                        ! When blood_samp_m2 is "true"...
CV_SAMPLE_M2  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m3 .xn. (2190-t)          ! At 3rd month of pregnancy, blood_samp_m3 is "true"
discrete blood_samp_m3                        ! When blood_samp_m3 is "true"...
CV_SAMPLE_M3  = Ca                            ! Record total maternal plasma level
end
```

```
schedule blood_samp_m4 .xn. (2920-t)          ! At 4th month of pregnancy, blood_samp_m4 is "true"
discrete blood_samp_m4                        ! When blood_samp_m4 is "true"...
CV_SAMPLE_M4  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m5 .xn. (3650-t)          ! At 5th month of pregnancy, blood_samp_m5 is "true"
discrete blood_samp_m5                        ! When blood_samp_m5 is "true"...
CV_SAMPLE_M5  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m6 .xn. (4380-t)          ! At 6th month of pregnancy, blood_samp_m6 is "true"
discrete blood_samp_m6                        ! When blood_samp_m6 is "true"...
CV_SAMPLE_M6  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m7 .xn. (5110-t)          ! At 7th month of pregnancy, blood_samp_m7 is "true"
discrete blood_samp_m7                        ! When blood_samp_m7 is "true"...
CV_SAMPLE_M7  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m8 .xn. (5840-t)          ! At 8th month of pregnancy, blood_samp_m8 is "true"
discrete blood_samp_m8                        ! When blood_samp_m8 is "true"...
CV_SAMPLE_M8  = Ca                            ! Record total maternal plasma level
end
schedule blood_samp_m9 .xn. (6569-t)          ! At 9th month of pregnancy, blood_samp_m9 is "true"
discrete blood_samp_m9                        ! When blood_samp_m9 is "true"...
CV_SAMPLE_M9  = Ca                            ! Record total maternal plasma level
CORD_SAMPLE   = Ca_f                          ! Record total cord plasma level
end

!-------------------------------------------------------------------------------------------
! Dosing parameters
Vol_dist = VPLASINIT+PG*VG+PL*VL &
           +PFat*VFATINIT+PK*VK+PR*VR &
           +PMam*VMamINIT+PSk*VSk                          ! Volume of distribution (L)
constant DOSE_INT = 1                                      ! Dose interval (h)
constant PFOS_HL  = 5.4                                    ! Half-life PFOS (years)
constant PFOA_HL  = 3.8                                    ! Half-life PFOA (years)
HALF_LIFE         = (PFOS*PFOS_HL+PFOA*PFOA_HL)*365*24     ! Half-life (h)
IVHOURLYDOSE      = CVINIT*Vol_dist*DOSE_INT/(1.44*HALF_LIFE)  ! Hourly dose (mg/h)

!-------------------------------------------------------------------------------------------
! Initial amounts of PFOS/PFOA in tissues (from initial blood level)
APLAS0  = CVINIT*VPLASINIT*Free    ! Amount free in plasma (mg)
AG0     = CVINIT*PG*VG             ! Amount in gut (mg)
AL0     = CVINIT*PL*VL             ! Amount in liver (mg)
AFAT0   = CVINIT*PFat*VFATINIT     ! Amount in fat (mg)
```

```
AK0     = CVINIT*PK*VK              ! Amount in kidney (mg)
AR0     = CVINIT*PR*VR              ! Amount in rest of body (mg)
AMAM0   = CVINIT*PMam*VMamINIT      ! Amount in mammary tissue (mg)
ASK0    = CVINIT*PSk*VSk            ! Amount in skin (mg)


!-----------------------------------------------------------------------------------------------
! MASS BALANCE DIFFERENTIAL EQUATIONS

!-- MOTHER --!

! FAT
RFat  = QFat*(CA*Free-CVFat*Free)   ! Rate of amount in fat (mg/h)
AFat  = integ(RFat,AFat0)           ! Amount in fat (mg)
CFat  = AFat/VFat                   ! Concentration in fat (mg/L)
CVFat = CFat/PFat                   ! Fat venous blood concentration (mg/L)

! MAMMARY TISSUE
RMam  = QMam*(CA*Free-CVMam*Free)   ! Rate of amount in mammary tissue (mg/h)
AMam  = integ(RMam,AMam0)           ! Amount in mammary tissue (mg)
CMam  = AMam/VMam                   ! Concentration in mammary tissue (mg/L)
CVMam = CMam/PMam                   ! Mammary tissue venous blood concentration (mg/L)

! GUT
RG  = QG*(CA*Free-CVG*Free)         ! Rate of amount in gut (mg/h)
AG  = integ(RG,AG0)                 ! Amount in gut (mg)
CG  = AG/VG                         ! Concentration in gut (mg/L)
CVG = CG/PG                         ! Gut venous blood concentration (mg/L)

! LIVER
RL  = (QL*(CA*Free)) &
      +(QG*CVG*Free) &
      -((QL+QG)*CVL*Free)           ! Rate of amount in liver (mg/h)
AL  = integ(RL,AL0)                 ! Amount in liver (mg)
CL  = AL/VL                         ! Concentration in liver (mg/L)
CVL = CL/PL                         ! Liver venous blood concentration (mg/L)

! KIDNEY
RK  = QK*(CA*Free-CVK*Free) &
      +(Tm*Cfil)/(Kt+Cfil)          ! Rate of amount in kidney (mg/h)
AK  = integ(RK,AK0)                 ! Amount in kidney (mg)
CK  = AK/VK                         ! Concentration in kidney (mg/L)
CVK = CK/PK                         ! Kidney venous blood concentration (mg/L)
```

```
! FILTRATE COMPARTMENT
RFil = Qfil*(CA*Free-Cfil) &
       -(Tm*Cfil)/(Kt+Cfil)          ! Rate of amount in filtrate compartment (mg/h)
AFil = integ(RFil,0.0)               ! Amount in filtrate compartment (mg)
Cfil = Afil/Vfil                     ! Concentration in filtrate compartment (mg/L)

! "DELAY" compartment for urine
Rdelay = Qfil*Cfil-kurine*Adelay     ! Rate of amount in "delay" compartment (mg/h)
Adelay = integ(Rdelay,0.0)           ! Amount in "delay" compartment (mg)

! URINE
RUrine = kurine*Adelay               ! Rate of amount excreted in urine (mg/h)
Aurine = integ(Rurine,0.0)           ! Amount excreted in urine (mg)

! SKIN
RSk  = QSk*(CA*Free-CVSk*Free)       ! Rate of amoune in skin (mg/h)
ASk  = integ(RSk,Ask0)               ! Amount in skin (mg)
CSk  = ASk/VSk                       ! Concentration in skin (mg/L)
CVSk = CSk/PSk                       ! Skin venous blood concentration (mg/L)

! REST OF BODY
RR  = QR*(CA*Free-CVR*Free)          ! Rate of amount in rest of body (mg/h)
AR  = integ(RR,AR0)                  ! Amount in rest of body (mg)
CR  = AR/VR                          ! Concentration in rest of body (mg/L)
CVR = CR/PR                          ! Rest of body venous blood concentration (mg/L0

! PLACENTA
RPla  = QPla*(CA*Free-CVPla*Free) &
        -rtrans1+rtrans2             ! Rate of amount in placenta (mg/h)
APla  = integ(RPla,0.0)              ! Amount in placenta (mg)
CPla  = APla/VPla                    ! Concentration in placenta (mg/L)
CVPla = CPla/PPla                    ! Placenta venous blood concentration (mg/L)

! PLASMA
RPlas    = (QFat*CVFat*Free) &
           +((QL+QG)*CVL*Free) &
           +(QR*CVR*Free) &
           +(QSk*CVSk*Free) &
           +(QK*CVK*Free) &
           +(QMam*CVMam*Free) &
           -(QC*CA*Free) &
           +(QPla*CVPla*Free) &
```

```
            +IVHOURLYDOSE &
            -Qfil*Ca*Free              ! Rate of amount free in plasma (mg/h)
APlas     = integ(RPlas,Aplas0)        ! Amount free in plasma (mg)
CAFree    = APlas/VPlas                ! Free concentration in plasma (mg/L)
CA        = CAFree/Free                ! Total concentration in plasma (mg/L)
APlastot  = CA*VPlas                   ! Amount total in plasma (mg)
AUCPlas   = integ(CA,0.0)              ! Area under the curve of Ca vs. time (mg*h/L)

IV_TOTAL = integ(IVHOURLYDOSE,0.0)     ! Total IV dose to the mother (mg)

!-- FETUS --!

! Transfer to fetus
rtrans1 = ktrans1*CVPla*Free           ! Transfer from placenta to fetal plasma (mg/h)
Atrans1 = integ(rtrans1,0.0)           ! Amount transferred to fetal plasma (mg)
rtrans2 = ktrans2*Ca_F*FreeF           ! Transfer from fetal plasma to placenta (mg/h)
Atrans2 = integ(rtrans2,0.0)           ! Amount transferred to placenta (mg)

! REST OF FETAL BODY
RRF = QRF*(CA_f*FreeF-CVRF*FreeF) &
      -rtrans3+rtrans4                 ! Rate of amount in fetal rest of body (mg/h)
ARRF = integ(RRF,0.0)                  ! Amount in fetal rest of body (mg)
CRF = ARRF/(VRF+1.0e-33)               ! Concentration in fetal rest of body (mg/L)
CVRF = CRF/PRF                         ! Fetal rest of body venous blood concentration (mg/L)

! AMNIOTIC FLUID
RAmF = rtrans3-rtrans4                 ! Rate of amount in amniotic fluid (mg/h)
AAmf = integ(RAmf, 0.0)                ! Amount in amniotic fluid (mg)
CAmf = AAmf/(Vaf+1.0e-7)               ! Concentration in amniotic fluid (mg/L)

rtrans3 = ktrans3*CVRF*FreeF           ! Transfer from fetal rest of body to amniotic fluid (mg/h)
Atrans3 = integ(rtrans3,0.0)           ! Amount transferred to amniotic fluid (mg)
rtrans4 = ktrans4*CAmF                 ! Transfer from amniotic fluid to fetal rest of body (mg/h)
Atrans4 = integ(rtrans4,0.0)           ! Amount transferred to fetal rest of body (mg)

! FETAL PLASMA
RPlasF    =(QRF*CVRF*FreeF) &
           -(QRF*CA_f*FreeF) &
           +rtrans1-rtrans2            ! Rate of amount in fetal plasma (mg/h)
APlasF    = integ(RPlasF,0.0)          ! Amount free in fetal plasma (mg)
CA_f_free = APlasF/(VPlasF+1.0e-7)     ! Free concentration in fetal plasma (mg/L)
CA_f      = CA_f_free/FreeF            ! Total concentration in fetal plasma (mg/L)
APlastotF = CA_f*VPlasF                ! Amount total in fetal plasma (mg)
```

```
AUCPLASF  = integ(CA_f,0.0)          ! Area under the curve Ca_f vs. time (mg*h/L)

!-------------------------------------------------------------------------------------------
! Dose balance check
BALANCE_DOSE = (IV_TOTAL+APLAS0+AG0+AL0+AFAT0+AK0+AR0+AMAM0+ASK0) &
               -(APLAS+AG+AL+AFAT+AK+AR+AMAM+ASK+APLA+APLASF+AFIL+ADELAY+AURINE+ARRF+AAMF)

!-------------------------------------------------------------------------------------------
! Stop the simulation
schedule stopthesim .xn. (TSTOP-t)  ! When TSTOP-simulation time becomes negative ...
discrete stopthesim                 ! Discrete event stopthesim is "true"
TERMT(.true.)                       ! When stopthesim is "true", stop the simulation
end

END ! Derivative
END ! DYNAMIC
END ! PROGRAM
```

```
% MODEL AUTOMATION SCRIPT FOR ACSLX (AEGIS TECHNOLOGIES GROUP, INC, HUNSTVILLE, AL, USA)

%-------------------------------------------------------------------------------------------%
%  Monte Carlo script for the PFAS_GESTATION_MC.csl model code                              %
%  Coded by Marc-Andre Verner                                                               %
%-------------------------------------------------------------------------------------------%

output @clear
prepare @clear @all

%-------------------------------------------------------------------------------------------%
% Turning off verbose diagnostic output
WESITG=0;
WXDITG=0;
WEDITG=0;
CJVITG=0;
WNDITG=0;

%-------------------------------------------------------------------------------------------
% Simulation parameters
TSTOP = 6570   ;
CINT  = 10000  ;
MINT  = 0.1    ;
MAXT  = 10000  ;
IALG  = 15     ;

sampled_values_MC_PFOS = [];
sampled_values_MC_PFOA = [];
VALUES_BIRTHWEIGHT_G   = [];

NUMITS = 250000;

seedrnd(123456789);

for x = 1 : NUMITS;

      disp(sprintf("Iteration #%d of %d", x, NUMITS));
      disp("---------------------------");

% Sensitive parameter distributions
PFOS_PL    = normrnd(3.72, 0.558, 2.604, 4.836)       ;
PFOA_PL    = normrnd(2.2, 0.33, 1.54, 2.86)           ;
PFOS_PR    = normrnd(0.2, 0.03, 0.14, 0.26)           ;
```

```
PFOA_PR    = normrnd(0.12, 0.018, 0.084, 0.156)       ;
PFOS_FREE  = normrnd(0.025, 0.00375, 0.01750, 0.03250);
PFOA_FREE  = normrnd(0.020, 0.00300, 0.00140, 0.02600);
PFOS_FREEF = normrnd(0.025, 0.00375, 0.01750, 0.03250);
PFOA_FREEF = normrnd(0.020, 0.00300, 0.00140, 0.02600);
PFOS_TMC   = normrnd(3.5, 0.525, 2.45, 4.55)          ;
PFOA_TMC   = normrnd(10.0, 1.5, 7.0, 13.0  )          ;
PFOS_KT    = normrnd(0.023, 0.00345, 0.0161, 0.0299)  ;
PFOA_KT    = normrnd(0.055, 0.00825, 0.0385, 0.0715)  ;
BWINIT     = normrnd(74.7, 17.8, 50.0, 114.0)         ;
VLC        = normrnd(0.026, 0.0039, 0.0182, 0.0338)   ;

% Calculation of birth weight (kg) based on GFR
RATIO_GFR     = normrnd(1.0, 0.246, 0.51, 1.49) ;
CONSTANT_REG  = 3.376 ;
BETA_REG      = 0.1755 ;
RESIDUAL      = normrnd(0.0, 0.441, -0.882, 0.882) ;
BIRTHWEIGHT   = BETA_REG*RATIO_GFR+CONSTANT_REG+RESIDUAL ;
BIRTHWEIGHT_g = BIRTHWEIGHT*1000 ;

%-------------------------------------------------------------------------------
% Simulations for PFOA
PFOS = 0 ;
PFOA = 1 ;

% Distibutions of blood levels in studies
AM    = 0.00253 ;
CV    = 0.446   ;
ASD   = AM*CV   ;
MIN   = 0.00001 ;
MAX   = 1.00000 ;

% Blood level parameters
CVINIT = normrnd(AM, ASD, MIN, MAX) ;

start @nocallback

% Record blood levels
initial_cv      = CVINIT;
cv_at_sample1   = CV_SAMPLE1 ;
cv_at_sample2   = CV_SAMPLE2 ;
cv_at_sample_m1 = CV_SAMPLE_M1 ;
cv_at_sample_m2 = CV_SAMPLE_M2 ;
```

```
cv_at_sample_m3 = CV_SAMPLE_M3 ;
cv_at_sample_m4 = CV_SAMPLE_M4 ;
cv_at_sample_m5 = CV_SAMPLE_M5 ;
cv_at_sample_m6 = CV_SAMPLE_M6 ;
cv_at_sample_m7 = CV_SAMPLE_M7 ;
cv_at_sample_m8 = CV_SAMPLE_M8 ;
cv_at_sample_m9 = CV_SAMPLE_M9 ;
cord_level      = CORD_SAMPLE ;
t_final         = T;

% Save PFOA results
if(abs(T-TSTOP)<1)
sampled_values_MC_PFOA = [sampled_values_MC_PFOA;  t_final cv_at_sample1 cv_at_sample2 cord_level ...
                          initial_cv PFOS_FREE PFOS_FREEF ...
                          PFOA_TMC PFOA_KT PFOA_K1C PFOA_K2C BWINIT RATIO_GFR RESIDUAL ...
                          cv_at_sample_m1 cv_at_sample_m2 cv_at_sample_m3 cv_at_sample_m4 ...
                          cv_at_sample_m5 cv_at_sample_m6 cv_at_sample_m7 cv_at_sample_m8 ...
                          cv_at_sample_m9 BIRTHWEIGHT_g];
end


%-------------------------------------------------------------------------------
% Simulations for PFOS
PFOS = 1 ;
PFOA = 0 ;

% Distibutions of blood levels in studies (from arithmetic means and stds)
AM    = 0.01302 ;
CV    = 0.368   ;
ASD   = AM*CV   ;
MIN   = 0.00001 ;
MAX   = 1.00000 ;

% Blood level parameters
CVINIT = normrnd(AM, ASD, MIN, MAX);

start @nocallback

% Record blood levels
initial_cv      = CVINIT;
cv_at_sample1   = CV_SAMPLE1 ;
cv_at_sample2   = CV_SAMPLE2 ;
cv_at_sample_m1 = CV_SAMPLE_M1 ;
```

```
cv_at_sample_m2 = CV_SAMPLE_M2 ;
cv_at_sample_m3 = CV_SAMPLE_M3 ;
cv_at_sample_m4 = CV_SAMPLE_M4 ;
cv_at_sample_m5 = CV_SAMPLE_M5 ;
cv_at_sample_m6 = CV_SAMPLE_M6 ;
cv_at_sample_m7 = CV_SAMPLE_M7 ;
cv_at_sample_m8 = CV_SAMPLE_M8 ;
cv_at_sample_m9 = CV_SAMPLE_M9 ;
cord_level      = CORD_SAMPLE ;
t_final         = T;

% Save PFOS results
if(abs(T-TSTOP)<1)
sampled_values_MC_PFOS = [sampled_values_MC_PFOS;  t_final cv_at_sample1 cv_at_sample2 cord_level ...
                          initial_cv PFOS_FREE PFOS_FREEF ...
                          PFOS_TMC PFOS_KT PFOS_K1C PFOS_K2C BWINIT RATIO_GFR RESIDUAL ...
                          cv_at_sample_m1 cv_at_sample_m2 cv_at_sample_m3 cv_at_sample_m4 ...
                          cv_at_sample_m5 cv_at_sample_m6 cv_at_sample_m7 cv_at_sample_m8 ...
                          cv_at_sample_m9 BIRTHWEIGHT_g];
end
end


save sampled_values_MC_PFOA @file=MC_PFOA_250K.csv @format=ascii @separator=comma
save sampled_values_MC_PFOS @file=MC_PFOS_250K.csv @format=ascii @separator=comma
```

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